ELSEVIER

Veterinary Parasitology 90 (2000) 171–181

veterinary parasitology

www.elsevier.com/locate/vetpar

Serologic survey of *Neospora caninum* infection in a closed dairy cattle herd in Maryland: risk of serologic reactivity by production groups

R.M. Dyer[a,b,*], M.C. Jenkins[c], O.C.H. Kwok[c], L.W. Douglas[d], J.P. Dubey[c]

[a] *Virginia/Maryland Regional College of Veterinary Medicine, University of Maryland Campus, 8075 Greenmeade Drive, College Park, MD 20742-3711, USA*

[b] *Department of Agricultural and Natural Resources, University of Delaware, Newark, DE 19717-1303, USA*

[c] *United States Department of Agriculture, Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory, Beltsville, MD 20705-2350, USA*

[d] *Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742-2311, USA*

Received 1 September 1998; accepted 30 March 2000

Abstract

Prevalence of antibodies to *Neospora caninum* was determined in a cross-sectional consensus survey of 1029 bovines in a dairy herd with endemic *Neospora*-induced abortion. Sera were screened by indirect fluorescent antibody test (IFAT). The prevalence of *N. caninum* antibody in the IFAT was 17.9% in 107 neonates, 26.2% in 233 yearling heifers and steers, 39.07% in 218 mature heifers, and 26.9% in 465 milking cows. Serologic reactivity was associated with production grouping on the farm with the greatest risk of serologic reactivity appearing in the yearling and mature heifers. There was an increasing risk of serologic reactivity with increasing age only in the parity one and greater animals in the herd. Castrated males were at half the risk of similarly aged females of possessing antibodies to *N. caninum*. There was no clear relationship between the serologic status of dams and offspring.

Keywords: *Neospora caninum*; Neosporosis; Abortion; Epidemiology; Dairy cattle; Bovine

1. Introduction

Neosporosis is now recognized as a major cause of abortion in cattle in many countries including the United States (reviewed in Dubey and Lindsay, 1996). Abortion can occur at any stage of pregnancy but most abortions occur at 5–6 months of gestation. Abortion may occur more than once in the same parity. Cows that aborted during a previous pregnancy due

* Corresponding author. Tel.: +1-301-935-6083; fax: +1-301-935-6079.
E-mail address: rd70@umail.umd.edu (R.M. Dyer)

0304-4017/00/$ – see front matter
PII: S0304-4017(00)00253-3

to neosporosis can abort again, or give birth to diseased calves, calves with a subclinical *Neospora caninum* infection, or calves that are not infected (Thilsted and Dubey, 1989; Anderson et al., 1991, 1995; Barr et al., 1993). Until recently, the full life cycle of *N. caninum* and sources of infection for cows were unknown. Transmission from the dam to the fetus is the predominant mode of natural *N. caninum* infection (Bjorkman et al., 1996; Paré et al., 1996; Anderson et al., 1997) even though a minor proportion of cattle seroconvert after birth (Paré et al., 1997; Thurmond et al., 1997; Davison et al., 1999; Hietala and Thurmond, 1999). Recently, dogs were shown to excrete resistant *N. caninum* oocysts in their feces after ingesting infected tissues (McAllister et al., 1998) and there presence on farms is a risk factor for *N. caninum* abortions (Paré et al., 1998; Bartels et al., 1999; Wouda et al., 1999). Canine derived oocysts are infective for bovine youngstock (De Marez et al., 1999).

Serologic surveys indicate widespread exposure to *N. caninum* in dairy and beef herds. However, most of the information is derived from cows from California (Conrad et al., 1993; Paré et al., 1995, 1996, 1997; Thurmond et al., 1997) or from surveys of small numbers or selected groups of cows (Thornton et al., 1994; Trees et al., 1994; Bjorkman et al., 1996; McAllister et al., 1996; Dubey et al., 1997; Thurmond et al., 1997). The main objective of the present study was to establish the seroprevalence of *N. caninum* in a large commercial dairy herd. We were particularly interested in surveying all cattle in this herd, irrespective of age, sex, and clinical history. We are planning to study the seroepidemiology of *N. caninum* in this herd over a period of many years with respect to prenatal and postnatal transmission. Results of the initial serologic survey are given in the present paper.

2. Materials and methods

The herd of 1029 cattle had been closed for more than 10 years and consisted of 113 calves (newborn–4 months), 280 heifers (4–20 months), 168 steers (4–24 months) and 465 parity one or greater milking cattle. All female replacement cattle were raised on the farm. All males were slaughtered at approximately 24 months of age. Animals were housed in four separate areas: neonatal calf facilities, young, female replacement and steer facilities, a mature female replacement facility, and the lactating herd facility. The facility housing male and female calves was within 100 m of the facility housing the mature, lactating herd. Facilities housing steers (approximately 4–24 months) and younger female replacement stock (approximately 4–12 months) were located 1600 m from the mature, milking herd. The older, female replacement stock (approximately 12–24 months) were housed 0.8 km from the main farm, the milking herd and all other young stock. Actual age ranges within each female group were: neonatal group 0.1–5.9 months, young female replacement group 3.4–13.6 months, older female replacement heifers 7.2–31.6 months and mature, lactating herd 23.9–163.8 months.

The lactating herd was housed in an open sided free stall barn attached by a central alleyway to the milking parlor. The milking herd was divided into five groups. These included cattle <1 month fresh, a second group producing >20 kg milk per day, a third group producing 15–19 kg milk per day, a fourth group producing <14 kg milk per day, and a fifth group consisting of first parity animals. All parity one and greater cattle were bred by

artificial insemination. The adult lactating herd remained separated from all other groups except when new-born calves exited the milk herd and newly freshened 2-year-old heifers entered the herd. All calves (newborn–4 months) were reared in individual calf hutches located in an open shed or converted stanchion barn. Calves received colostrum within the first 6 h of birth. For the first 4 months of age, the diet consisted of milk replacer, discarded milk, commercial calf starter concentrate and mixed grass hay. Calves 4 months of age were assembled into groups of 20–40 animals and housed in two pole barns, located adjacent to one another with access to a common cement apron and feedbunk. Within this site, animals were segregated by gender and placed in one of two pole barns. Young stock remained segregated by gender but feed, bedding, manure disposal and labor were shared between populations. All steers remained in this facility until slaughter weight (500 kg in 18–24 months). Younger replacement females remained in the pole barn until approximately 10–12 months of age. These yearling females were moved to another farm located 0.8 km from the main farm, the milking herd and all other young stock. In contrast to parity one and older animals, these mature replacement heifers were naturally inseminated with 4–6 bulls allowed to roam freely with the females. After conception, females remained at this site until parturition at 22–24 months.

The rations were total mixed rations consisting of corn silage, alfalfa haylage, ryelage, various feed commodities and mineral supplements. All cattle groups were fed in open feed bunks. All roughages were stored in trench silos and feed commodities were stored in compartmentalized, open front sheds. Feed storage and ration assembly was located in a single central facility. Rations were distributed from the single facility to each production group.

The herd had a 25-year history of undiagnosed, chronic, endemic abortion. Pregnancy status was always determined by rectal examination, and a diagnosis of abortion was assigned when a confirmed pregnancy was no longer palpable at some later date. In 1994, 237 of 465 cows were confirmed pregnant. Of the pregnant cows 54 or 18.6% aborted with a mean gestation (±S.D.) of 116.7 (59.7) days. Thirty-six of the aborted cattle were bred again but four re-aborted (11.1%) in the same parity. In the fall of 1994, a 3-day abortion episode affected 13 bred heifers located in the mature heifer population. *N. caninum* was identified by histologic examination and the diagnosis was confirmed by immuno-histochemistry (Anderson et al., 1991) in an aborted fetus. The epicardium and subjacent myocardium of several hearts contained moderate to severe mononuclear cell and mild neutrophilic infiltrates. Sections of brain contained multifocal areas of necrosis and gliosis. These lesions were consistent with abortion due to *N. caninum*. Further, immunohistochemical evaluation of brain sections from one calf revealed tissue cysts that stained positive for *N. caninum*. During the same period, 38 paired sera showed no evidence of serologic conversion to infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, *Leptospira canicola*, *Leptospira grippotyphosa*, *Leptospira hardjo*, *Leptospira icterohaemmorrhagiae* or *Leptospira pomonna*. Virus and bacterial examination of all fetuses failed to reveal a causative agent. Blood cultures from the entire herd and all calves born up to 7 months after the initial survey were negative for BVD virus upon microtiter plate culture. The herd was on a biannual vaccination program against infectious bovine rhinotracheitis virus, bovine viral diarrhea virus, parainfluenza three virus and *Leptospira* species.

An aged, mixed breed, female dog lived on the farm and had access to all animals and feed storage facilities.

2.1. Serologic evaluation

Blood samples were obtained from the entire herd (1029 cattle) over a period of 3 days. Sera were separated and stored at −20°C until tested for *N. caninum* antibodies. An indirect fluorescent antibody test (IFAT) was used to evaluate sera for *N. caninum* antibodies. For IFAT, whole tachyzoite smears and anti-bovine conjugates were obtained commercially (VMRD, Pullman, WA, USA). Sera were diluted 1:200 with phosphate buffer saline (PBS), pH 7.2 and examined for whole tachyzoite fluorescence as described (Paré et al., 1995). We selected a single dilution of 1:200 as indicative of *N. caninum* exposure based on past experience, although there are no definitive data concerning an IFAT titer that should be regarded as indicative of persistent *N. caninum* infection in cattle (Dubey and Lindsay, 1996; Dubey et al., 1997).

2.2. Dam–daughter pairings

Family trees were constructed for all female cattle in the herd at the time of the cross-sectional consensus survey. Dam–daughter relationships were established from individual Dairy Herd Improvement Association (DHIA) cow sheets and confirmed from a second set of records containing birth date, dam identification and assigned herd number of female offspring. Where more than one daughter was present for a dam, one daughter was selected using the random number generator. Vertical transmission was calculated as the number of positive daughters born to positive dams divided by the total number of positive dams.

2.3. Statistical methods

Two logistic analysis were performed using the generalized linear mixed macro of SAS, Version 6.12. One analysis modeled the affects of gender and facility, while the second analysis modeled the affects of facility and resident age (time spent in the facility). For both analysis, the error distribution was defined as binomial and the link was defined as ‘logit’, thus, specifying logistic analysis. The IFAT status was the response variable and was coded as a dichotomous variable (0=negative, 1=positive) for each animal. Six groups were defined as the explanatory variable based on gender (male or female) and facility (four locations). The groups were: (1) female calves in the neonatal facility, (2) male calves in the neonatal facility, (3) adolescent heifers in the pole barn, (4) steers in the pole barn, (5) heifers in the mature heifer facility, and (6) parity one or greater cows located in the freestall and parlor facility.

The first analysis included all six groups with selected mean contrasts to address hypotheses of interest. These included facility comparisons within gender, gender comparisons within facilities, the interaction comparison of gender and facility at the neonatal and pole barn facilities, and a gender comparison for 4–24 month old, similarly aged heifer and steers. The mean IFAT for each group represents prevalence and is equal to the number

of positive animals divided by the total number of animals at risk. Relative risk (RR) was also computed as e', where '' is the mean log odds ratio for group 1 minus the mean log odds ratio for group 2. Group 1 is the reference group because female calves in the neonatal facility had the lowest seropositivity of *N. caninum* for all RR calculations in the current manuscript.

The second analysis included a variable based on age as an indicator of the length of time spent by the animal in the facility. Age in months (rounded to the nearest 10th of a month) was available only for females, thus this analysis included only the four female groups. Because age and facility are confounded, resident age was compounded as age minus mean age of females in the facility, thus since animals were moved to a facility at approximately the same age, resident age is a measure of the time spent in the facility. An animal with a zero resident age would have been housed in the facility for a length of time equal to the average of females in the facility. An animal with a resident age of −4, for example, would have been housed in the facility 4 months less than the average of females in the facility. Exploratory variables in this analysis included the four groups as a class variable and resident age (linear) and resident age squared (quadratic) as continuous variables. Also included in the model was the interaction between facilities and resident age. Thus, for each facility, the model examined the relationship between seropositivity and the length of time spent in the facility.

Two, 2×2 contingency table analysis were conducted using Fisher's exact test to evaluate the dam–daughter association (Schlesselman, 1982; Kleinbaum et al., 1988). The first analysis included all available dam–daughter pairs available in the herd, while the second included only those dam–daughter pairs where the daughter was less than first parity.

3. Results

3.1. Serologic prevalence

Seropositivity of *N. caninum* in the consensus survey is reported in Table 1. Overall, 28.0% of the herd was seropositive for *N. caninum*. In this herd, 20.6% (95% C.I., 12.6–31.9) of

Table 1
Cross-sectional survey of *N. caninum* antibodies in a commercial dairy herd in Maryland

Group location	Age (months)	No. of animals	Seropositive by IPAT (%)	Relative risk (95% C.I.)
Calf hutches		113	17.9	
Female	1.9 (1.0)[a]	68	20.6	1.0
Male	NA	45	15.6	0.7 (0.3–1.9)
Off-site pole		233	26.2	
Barns				
Female	8.3 (1.9)[a]	62	30.7	1.7 (0.8–3.8)
Male	NA	171	22.2	1.1 (0.6–2.2)
Off-site mature				
Heifer farm	16.7 (4.5)[a]	218	39.0	2.5 (1.3–4.5)
Freestall with parlor (lactating herd)	50.7 (23.5)[a]	465	26.9	1.4 (8–2.6)

[a] Mean: (S.D.).

heifer calves in the neonate facility, 30.7% (20.4–43.2) of adolescent heifers in the pole barn, 39.0% (32.7–45.7) of mature heifers, and 26.9% (23.0–31.1) of milking cows had antibodies for *N. caninum*. There were differences among females in different facilities (p=0.0045), with the prevalence being significantly greater at the mature heifer facility than at the neonate facility or the milking cow facility. No other facility differences were significant for females. The relative risk of possessing antibodies to *N. caninum* was 2.5 (95% C.I., 1.3–4.7) times greater at the mature heifer facility than the females at the neonate facility: 1.7 (0.8–3.8) times greater for adolescent heifers in the pole barn than for females at the neonate facility; and 1.4 (0.8–2.6) times greater at the milking cow facility than for the females at the neonate facility. The prevalence of *N. caninum* in males was 15.6% (95% C.I., 7.6–29.3) at the neonate facility and 21.8% (16.2–28.6) at the pole barn, which was not significantly different. The prevalence of *N. caninum* in males and adolescent females at the pole barn was 21.8% (16.2–28.6) and 30.7% (20.4–43.2), respectively, which was not significantly different.

The relative risk of possessing antibodies to *N. caninum* was 0.7 (0.3–1.9) times less for male calves than for female calves in the neonate facility and 1.1 (0.6–2.2) times greater for male steers in the pole barn than for female calves in the neonate facility. Neither of these differences, nor the interaction effect of gender and facility were significant at these two facilities. Because heifers were moved to the mature heifer facility at 10–12 months of age, while steers remained in the pole barn facility until slaughter, this comparison did not compare similarly aged groups of males and females. A comparison between similarly aged, but not facilities matched gender groups, can be obtained by combining data from females in both the pole barn and mature heifer barn (4–24 months of age) and comparing this to the males in the polebarn (4–24 months of age). This comparison resulted in a significantly greater seropositivity (p=0.010) for heifers of 34.7% (95% C.I., 28.2–41.9) and for steers of 22.2% (95% C.I., 6.6–29.1).

The second analysis evaluated the association between seropositivity and resident age (months spent in the facility) for females. The final model included a common quadratic

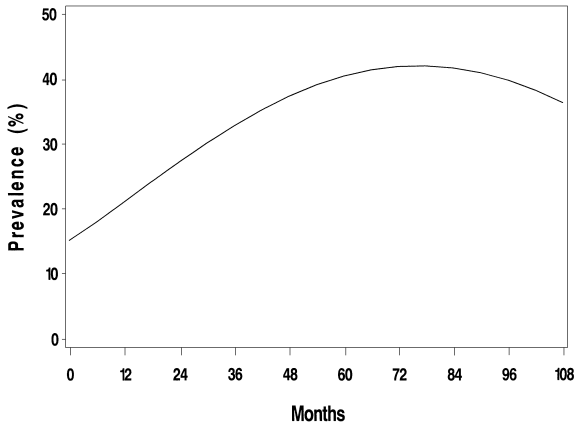

Fig. 1. Prevalence of *N. caninum* in parity one and greater cattle of a closed, endemically infected dairy herd increases with longevity in the lactating population.

Table 2
Cross-classification of dam–daughter pairings by IFAT determined *N. caninum* serologic reactivity in daughters of any age

		Daughter		
		Negative	Positive	Total
Dam	Negative	120	34	154
	Positive	54	35	89
	Total	174	69	243

component and a different linear relationship between prevalence and adjusted age at the facility. Within the three heifer facilities (neonate, adolescent and mature) seropositivity was lower for older heifers than for younger heifers, although none of these relationships were significant. Only at the milking cow facility was there a significant relationship with age (p=0.0008) and that was a positive nonlinear increase in prevalence with increasing longevity in the lactating population (Fig. 1). The predicted prevalence was approximately 15% for 24 month old first parity cows and increased at a rate of about 0.5% per month of age to approximately 60 months of age (Fig. 1). The rate of increase in prevalence after 60 months slowed with prevalence peaking at about 41% for cows between 84 and 108 months. After the 108 months predicted prevalence declined slightly, but this portion of the curve should be considered with caution since less than 5% of the cows were older than 101 months of age.

3.2. Dam–daughter pairings

Sufficient serologic data were available to construct 243 dam–daughter pairings (Table 2) involving daughters of any age. Vertical transmission to female offspring of all ages was 39.3%. The percent positive dams associated with positive offspring (14.4%) was similar to the percent negative dams associated with positive offspring (14.0%). Serologic status of the dam was significantly related to serologic status of the daughter (p=0.005) in that negative dams were 2.29 (95% C.I., 1.243–4.215) more likely to have negative offspring as positive dams.

Data were also examined for 229 dam–daughter pairings (Table 3) involving daughters less than first parity. Vertical transmission to female offspring 1 year or less in age was

Table 3
Cross-classification of dam–daughter pairings by IFAT determined *N. caninum* serologic reactivity in daughters less than parity one

		Daughter		
		Negative	Positive	Total
Dam	Negative	108	47	155
	Positive	42	32	74
	Total	150	79	229

43.2%. Serologic status of the dam was significantly related to serologic status of the daughter (p=0.05) in that negative dams were 1.751 (95% C.I., 0.948–3.234) more likely to have negative offspring as positive dams.

4. Discussion

Four findings distinguish the presentation of neosporosis in this herd from that described in other herds. (1) The present herd experienced nearly half the rate of congenital infection of calves from seropositive dams compared to that in other herds. (2) There was an increased risk of serologic prevalence in the 12–24-month-old females located in the offsite, mature heifer facility, compared to females located in any other facility. (3) There was a 0.5% increase in prevalence with each successive month of a cow's tenure in the lactation facility. (4) Males possessed half the risk of being seropositive for *N. caninum* as similarly aged females.

Vertical transmission in this herd (39–43%) was considerably lower than 81.0, 83.8 and 88.2% vertical transmission previously reported in other reports (Paré et al., 1995, 1996, 1997). The explanation for the differences is not clear. Unknown host, parasite or environmental events may be affecting in utero exposure and/or transmission. The technique of sera sampling may account for some of the differences. Determining serologic status on pre-colostrum blood (Paré et al., 1995, 1996, 1997) controls for factors such as morbidity and deterioration in titers that might accompany the postcolustral sampling method employed in this survey. Unlike an earlier report, we found a significant association between serologic status of dams and daughters (Waldner et al., 1998). The strength of the association arose more from the association of negative dams with negative daughters rather than positive dams with positive daughters. Similar to data from other herds, a high number of the dam–daughter pairs in this herd consisted of negative dams matched with positive daughters (Paré et al., 1995, 1996). These pairings could arise as some daughters become seroreactive after postnatal exposure or antibody levels in dams decline below the sensitivity of the IFAT (Hietala and Thurmond, 1999). In the former case, the incidence of postnatal seroconversion in this herd must be low or the significant association between the serologic status of the dams and daughters would not exist. Earlier associations between negative dams and positive calves were attributed to a low diagnostic sensitivity of the IFAT performed at a 1:640 serum dilution (Paré et al., 1995). Use of a 1:200 serum dilution in our screen should improve, but may have not eliminated this concern (Dubey et al., 1997). In addition, other data suggests antibody titers in chronically infected adult cattle may rise, fall or temporarily deteriorate below test sensitivity (Conrad et al., 1993; Dubey et al., 1996, 1997; Jenkins et al., 1997; Hietala and Thurmond, 1999). Consequently, an undetermined number of negative IFAT titers in this cross-sectional survey may represent undetectable antibodies in otherwise, chronically infected dams. This might explain why apparently negative dams were paired with positive daughters and predicts these dams will show detectable antibody levels in a prospective study (Hietala and Thurmond, 1999). The potential for low diagnostic sensitivity in the IFAT warrants a cautious interpretation at this time.

The increased risk of seropositivity in the mature female heifers in this herd extends findings in other herds (Paré et al., 1996; Waldner et al., 1998) where the relationship

between age and prevalence was evaluated in adult animals. The higher risk of seropositivity in the mature heifer groups, coinciding with a relatively lower prevalence in the neonatal and parity one and greater animal groups, could be indirect evidence of postnatal infection or exposure in the mature heifer facility. Other evidence in favor of postnatal *N. caninum* exposure are spontaneous outbreaks of neosporosis-abortion in cattle (Thornton et al., 1994; Yeager et al., 1994; Moen et al., 1995; McAllister et al., 1996) and isolated serologic responses to *N. caninum* in 18% of otherwise negative cattle (Hietala and Thurmond, 1999). In accordance with the former reports, an explosive outbreak of abortion, associated with the presence of a *N. caninum* encephalitis was experienced 8 months before this survey by the same mature heifer group found at greatest risk of possessing antibodies to *N. caninum*. Data from our survey support a model of neospora infection wherein an externally derived, high-dose infection suddenly elevates herd prevalence (French et al., 1999). Any other time, prevalence is sustained by a high fidelity of vertical transmission, minimal fetal loss and low rates of horizontal transmission (Paré et al., 1997; Thurmond et al., 1997; Davison et al., 1999; Hietala and Thurmond, 1999).

A nonlinear, positive relationship of prevalence with animal tenure in the lactating herd facility has not been described. Others found no correlation between age and serologic reactivity in another herd of dairy cattle (Paré et al., 1996). In beef, older cows were at less risk for possessing antibodies to *N. caninum* (Waldner et al., 1998). There are several plausible explanations for our findings. Possibly the relationship reflects the herd's history with neospora. The low fidelity of vertical transmission in this herd could be expected to diminish neospora prevalence with successive generations of replacement stock introduced into the lactating herd (Fine, 1975; French et al., 1999). Accordingly, animals with the longest tenure in the lactating facility would show the highest prevalence and cattle with the shortest tenure would possess the lowest prevalence. Alternatively, sporatic expression of serologic reactivity in seronegative cows has been interpreted as postnatal exposure without infection (Hietala and Thurmond, 1999). The increasing prevalence with increasing tenure in the lactating facility in our herd could reflect a greater opportunity of exposure and seroconversion in older animals.

The castrated males in the herd were at significantly less risk of having antibodies against *N. caninum* than all the similarly aged females in the herd. There were no differences between the prevalence in castrated males and the 4–12-month-females located in the pole barn facility. Accordingly, prevalence differences between similarly aged males and females likely arose from the relatively high prevalence in the 12–24-month-old females. These animals lived in an offsite facility located some distance from the pole barn that housed the 4–24-month-old males and the 4–12-month-old females. Opportunities for intermittent exposure to *N. caninum* should be the same across genders. The only way exposure could be biased against one sex is if the sexes were separated into different facilities and facility exposure was biased. Accordingly, one could speculate exposure was biased against the facility housing the 12–24 month heifers. Possibly, selective oocyst contamination of this female environment by the resident farm dog could explain the finding (McAllister et al., 1998; Paré et al., 1998). Otherwise, one would have to postulate that castrated males were protected against infection but not exposure.

Models of prevalence dynamics of infectious diseases indicate vertical transmission alone cannot sustain endemicity if host reproductive fecundity is reduced and/or the fidelity of

vertical transmission is below 100% (Fine, 1975; Lipstitch et al., 1995; French et al., 1998). With an abortion rate of 18%, and a 39.3% rate of vertical transmission in this herd, the models predict the prevalence of neosporosis should fall to zero unless some horizontal transmission occurs. This trend may be evident in the lactating herd. The high risk of seropositivity in the young females may have evolved from an episode of horizontal transmission predicted to sustain neospora endemicity (Fine, 1975; French et al., 1999). This issue needs further clarification by prospective studies.

Acknowledgements

This work was supported in part by a grant from the University of Maryland Agricultural Experiment Station.

References

Anderson, M.L., Blanchard, P.C., Barr, B.C., Dubey, J.P., Hoffman, R.L., Conrad, P.A., 1991. *Neospora*-like protozoan infection as a major cause of abortion in California dairy cattle. J. Am. Vet. Med. Assoc. 198, 241–244.

Anderson, M.L., Palmer, C.W., Thurmond, M.C., Picanso, J.P., Blanchard, P.C., Breitmeyer, R.E., Layton, A.W., McCallister, M., Draft, B., Kindle, H., Read, D.H., Dubey, J.P., Conrad, P.A., Barr, B.C., 1995. Evaluations of abortions in cattle attributable to neosporosis in selected dairy herds in California. J. Am. Vet. Med. Assoc. 207, 1206–1210.

Anderson, M.L., Reynolds, J.P., Rowe, J.D., Sverlow, K.W., Packham, A.E., Barr, B.C., Conrad, P.A., 1997. Evidence of vertical transmission of *Neospora* sp. infection in dairy cattle. J. Am. Vet. Med. Assoc. 210, 1169–1172.

Barr, B.C., Conrad, P.A., Breitmeyer, R., Sverlow, K., Anderson, M.L., Reynolds, J., Chauver, A.E., Dubey, J.P., 1993. Congenital *Neospora* infection in calves born from cows that had previously aborted *Neospora*-infected fetuses: four cases (1990–1992). J. Am. Vet. Med. Assoc. 202, 113–117.

Bartels, C.J.M., Wouda, W., Schukken, Y.H., 1999. Risk factors for *Neospora caninum* associated abortion storms in dairy herds in The Netherlands (1995–1997). Theriogenology 52, 247–252.

Bjorkman, C., Johansson, O., Stenlund, S., Holmdahl, O.J., Uggla, A., 1996. *Neospora* species infection in a herd of dairy cattle. J. Am. Vet. Med. Assoc. 208, 1441–1444.

Conrad, P.A., Sverlow, K., Anderson, M.L., Rowe, J., BonDurant, R., Tuter, G., Breitmeyer, R., Palmer, J., Thurmond, M., Ardans, A., Dubey, J.P., Duhamel, G., Barr, B., 1993. Detection of serum antibody responses in cattle with natural or experimental *Neospora* infection in dairy cattle. Int. J. Vet. Diagn. Invest. 5, 572–578.

Davison, H.C., Otter, A., Tree, A.J., 1999. Estimation of vertical and horizontal transmission parameters of *Neospora caninum* infection in dairy cattle. Int. J. Parasitol. 29, 1683–1689.

De Marez, T., Liddell, S., Dubey, J.P., Jenkins, M.C., Gasbarre, L., 1999. Oral infection of calves with *Neospora caninum* oocysts from dogs: humoral and cellular immune responses. Int. J. Parasitol. 29, 1647–1657.

Dubey, J.P., Jenkins, M.C., Adams, D.S., McAllister, M.M., Anderson, M.L., Sprecher, R., Baszler, T.V., Kwok, O.C.H., Lally, N.C., Bjorkman, C., Uggla, A., 1997. Antibody responses of cows during an outbreak of neosporosis evaluated by indirect fluorescent antibody test and different enzyme linked immunosorbent assays. J. Parasitol. 83, 1063–1069.

Dubey, J.P., Lindsay, D.S., 1996. A review of *Neospora caninum* and neosporosis. Vet. Parasitol. 67, 1–59.

Dubey, J.P., Lindsay, D.S., Adams, J.M., Gay, T.V., Baszler, D.S., Blagburn, B.L., Thulliez, P., 1996. Serologic responses of cattle and other animals infected with *Neospora caninum*. Am. J. Vet. Res. 57, 329–336.

Fine, P.E., 1975. Vectors and vertical transmission: an epidemiologic perspective. Ann. N. Y. Acad. Sci. 266, 173–194.

French, N.P., Davidson, H.C., Clancy, D., Begon, M., Trees, A.J., 1998. Modeling of *Neospora caninum* species infection in dairy cattle: the importance of horizontal and vertical transmission and differential culling. In: Proceedings of the Society of Veterinary Epidemiology and Preventive Medicine, Ennis, 25–27 March 1998, pp. 113–122.

French, N.P., Clancey, D., Davison, H.C., Trees, A.J., 1999. Mathematical models of *Neospora caninum* infection in cattle: transmission and options for control. Int. J. Parasitol. 29, 1691–1704.

Jenkins, M.C., Wouda, W., Dubey, J.P., 1997. Serologic responses overtime to recombinant *Neospora caninum* antigens in cattle after a neosporosis-induced abortion. Clin. Diagn. Lab. Immunol. 4, 270–274.

Hietala, S.K., Thurmond, M.C., 1999. Postnatal *Neospora caninum* transmission and transient serologic response in two dairies. Int. J. Parasitol. 29, 1669–1676.

Kleinbaum, D.G., Kupper, L.L., Muller, K.E., 1988. Applied Regression Analysis and Other Multivariable Methods. Prindle, Weber & Schmidt, Boston, MA.

Lipstitch, M., Nowak, M.A., Ebert, D., May, R.M., 1995. The population dynamics of vertically and horizontally transmitted parasites. Proc. R. Soc. Lond. B. 260, 321–327.

McAllister, M.M., Dubey, J.P., Lindsay, D.S., William, R.J., Wills, R.A., McGuire, A.M., 1998. Dogs are definitive hosts of *Neospora caninum*. Int. J. Parasitol. 28, 1473–1478.

McAllister, M.M., Huffman, E.M., Hietala, S.K., Conrad, P.A., Anderson, M.L., Salman, M.O., 1996. Evidence suggesting a point source exposure in an outbreak of bovine abortion due to neosporosis. J. Vet. Diagn. Invest. 8, 355–357.

Moen, A.R., Wouda, W., van Werven, T., 1995. Clinical and sero-epidemiological follow-up study in four dairy herds with an outbreak of *Neospora* abortion. In: Proceedings of the Dutch Society for Veterinary Epidemiology and Economics, Lelystad, 13 December 1995, pp. 93–103.

Paré, J., Fecteau, G., Fortin, M., Marsolais, G., 1998. Seroepidemiologic study of *Neospora caninum* in dairy herds. J. Am. Vet. Med. Assoc. 11, 1595–1598.

Paré, J., Thurmond, M.C., Hietala, S.K., 1995. Interpretation of an indirect fluorescent antibody test for diagnosis of *Neospora* sp. infection in cattle. J. Vet. Diagn. Invest. 7, 273–275.

Paré, J., Thurmond, M.C., Hietala, S.K., 1996. Congenital *Neospora caninum* infection in dairy cattle and associated calfhood mortality. Can. J. Vet. Res. 60, 133–139.

Paré, J., Thurmond, M.C., Hietala, S.K., 1997. Neospora *caninum* antibodies in cows during pregnancy as a predictor of congenital infection and abortion. J. Parasitol. 83, 82–87.

Schlesselman, J., 1982. Case-Control Studies. Oxford University Press, New York.

Thilsted, J.P., Dubey, J.P., 1989. Neosporosis-like abortions in a herd of dairy cattle. J. Vet. Diagn. Invest. 1, 205–209.

Thornton, R.N., Gajadhar, A., Evans, J., 1994. Neospora abortion epidemic in a dairy herd. New Zealand Vet. J. 42, 190–191.

Thurmond, M.C., Hietala, S.K., Blanchard, P.C., 1997. Herd-based diagnosis of *Neospora caninum*-induced endemic and epidemic abortion in cows and evidence for congenital and postnatal transmission. J. Vet. Diagn. Invest. 9, 44–49.

Trees, A.J., Guy, F., Low, J.C., Roberts, L., Buxton, D., Dubey, J.P., 1994. Serological evidence implicating *Neospora* species as a cause of abortion in British cattle. Vet. Rec. 134, 405–407.

Waldner, C.L., Janzen, E.D., Ribble, C.S., 1998. Determination of the association between *Neospora caninum* infection and reproductive performance in beef herds. J. Am. Vet. Med. Assoc. 213, 685–690.

Wouda, W., Dijkstra, T., Kramer, A.M.H., van Maanen, C., Brinkhof, J.M.A., 1999. Seroepidemiological evidence for a relationship between *Neospora caninum* infection in dogs and cattle. Int. J. Parasitol. 29, 1677–1682.

Yeager, M.J., Shawd-Wessels, S., Leslie-Steen, P., 1994. *Neospora* abortion storm in a mid-western dairy. J. Vet. Diagn. Invest. 6, 506–508.